Research Article

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CD147 reprograms fatty acid metabolism in hepatocellular carcinoma cells through Akt/mTOR/SREBP1c and P38/PPARα pathways

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Background & Aims: CD147 is a transmembrane glycoprotein which is highly expressed in various human cancers including hepatocellular carcinoma (HCC). A drug Licartin developed with ^{131}Iodine-labeled antibody against CD147 has been approved by the Chinese Food and Drug Administration (FDA) and enters into clinical use for HCC treatment. Increasing lines of evidence indicate that CD147 is implicated in the metabolism of cancer cells, especially glycolysis. However, the molecular mechanism underlying the relationship between CD147 and aberrant tumor lipid metabolism remains elusive.

Methods: We systematically investigated the role of CD147 in the regulation of lipid metabolism, including *de novo* lipogenesis and fatty acid β-oxidation, in HCC cells and explored the underlying molecular mechanisms.

Results: Bioinformatic analysis and experimental evidence demonstrated that CD147 significantly contributed to the reprogramming of fatty acid metabolism in HCC cells mainly through two mechanisms. On one hand, CD147 upregulated the expression of sterol regulatory element binding protein 1c (SREBP1c) by activating the Akt/mTOR signaling pathway, which in turn directly activated the transcription of major lipogenic genes *FASN* and *ACC1* to promote *de novo* lipogenesis. On the other hand, CD147 downregulated peroxisome proliferator-activated receptor alpha (PPARα) and its transcriptional target genes *CPT1A* and *ACOX1* by activating the p38 MAPK signaling pathway to inhibit fatty acid β-oxidation. Moreover, *in vitro* and *in vivo* assays indicated that the CD147-mediated reprogramming of fatty acid metabolism played a critical role in the proliferation and metastasis of HCC cells.

Conclusion: Our findings demonstrate that CD147 is a critical regulator of fatty acid metabolism, which provides a strong line of evidence for this molecule to be used as a drug target in cancer treatment.

Introduction

Recently, alteration of lipid metabolism has been increasingly recognized as a hallmark of cancer cells [1]. In contrast to normal cells using exogenous fatty acids, many cancer cells show high rates of *de novo* lipid synthesis. Continuous *de novo* lipogenesis provides cancer cells with membrane building blocks, signaling lipid molecules and post-translational modifications of proteins to support rapid cell proliferation [1,2]. Furthermore, the expression and activity of many key enzymes involved in *de novo* fatty acid synthesis, such as ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), are upregulated and associated with poor clinical outcomes in many types of cancers [3–5]. However, mechanisms underlying the increased lipogenesis in cancers are not completely understood. With most current research focusing on lipogenesis, the relevance of fatty acid oxidation (FAO) to cancer cells has received less attention. Recently, the importance of FAO in cancer metabolism is being increasingly recognized. Carnitine palmitoyltransferase 1 (CPT1) is a key regulatory enzyme in FAO and has been extensively studied as a potential therapeutic target for the treatment of metabolic syndromes [6,7]. A few recent reports also implicated CPT1 in regulating apoptosis and cancer development [8]. Peroxisomal acyl-coenzyme aoxidase 1 (ACOX1) is another key enzyme of the FAO pathway. Fan *et al.* reported that the older *Acox1*$^{(-/-)}$

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Abbreviations: HCC, hepatocellular carcinoma; FASN, fatty acid synthase; ACC, Acetyl-CoA carboxylase; SREBP1c, sterol regulatory elementary binding protein1c; Akt, protein kinase B; mTOR, mammalian target of rapamycin; CPT1A, carnitine palmitoyltrans-ferase 1A; ACOX1, acyl-coenzyme aoxidase 1; PPAR, peroxisome proliferation activated receptor; MAPK, mitogen-activated protein kinase.

mice developed hepatocellular carcinomas (HCC) [9]. These findings suggested a critical role of the FAO pathway in tumorigenesis. However, despite the identification of several intracellular signaling mediators in the regulation of lipid metabolism, it remains largely unclear how cells cope with metabolic stress and convert extracellular stimuli into intracellular signals. To answer these questions, it is necessary to investigate the role of transmembrane proteins in reprogramming of lipid metabolism.

CD147, a transmembrane glycoprotein, is highly expressed on the surface of various malignant cells including HCC [10,11]. A series of previous studies demonstrated that the upregulated CD147 expression contributes significantly to tumor growth, metastasis and angiogenesis [12,13]. Moreover, a radioimmunotherapeutic drug Licartin (generic name, [^{131}I] metuximab injection), which was generated by labeling ^{131}Iodine onto murine monoclonal antibody against CD147, has been previously approved by Chinese Food and Drug Administration (FDA) to treat HCC [14,15]. A wide array of studies have indicated that CD147 mainly functions as a cellular adhesion molecule and is important in cell-cell and cell-extracellular matrix interactions that induce the secretion of matrix metalloproteinases [16]. More strikingly, CD147 has also been described to play a pivotal role in the regulation of cell metabolism [11,17]. Recently, we have demonstrated that CD147 significantly contributes to the reprogramming of glucose metabolism in HCC cells through a p53-dependent way [18]. However, to date, the molecular mechanism underlying the relationship between CD147 and aberrant tumor lipid metabolism remain elusive.

Given the crucial role played by lipid metabolism in cancer development, it is important to identify new molecules and pathways that reprogram this process. In the present study, we systematically explored the role of CD147 in the reprogramming of fatty acid metabolism in HCC cells.

Materials and methods

Antibodies and reagents

The primary antibodies used in this study and their working concentration are listed in Supplementary Table 1. The PI3K inhibitor LY294002 was purchased from Cell Signaling Technology Inc (Beverly, MA). Peroxisome proliferator-activated receptor alpha (PPARα) inhibitor MK886 and agonist GW7647 were purchased Sigma-Aldrich (St. Louis, MO), and Abcam (Cambridge, MA). p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 was purchased from Selleckchem (Houston, TX).

Collection of public dataset and bioinformatic analysis

Four public datasets of mRNA expression data in HCC tissues were analyzed, including RNA-seq data from The Cancer Genome Atlas and microarray data of GSE36376, GSE25097 and GSE22058 from Gene Expression Omnibus database. The detailed information is listed in Supplementary Table 2. Expression correlation between CD147 and other genes were determined using Spearman correlation analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the online DAVID tool (http://niaid.abcc.ncifcrf.gov/) was performed as previously described [19].

Cell lines and tissue samples

Human HCC cell lines SMMC-7721 and MHCC97L were routinely cultured. SMMC-7721 cell lines which differ only in their CD147 status have been previously established [18]. Similarly, MHCC97L cell with stable knockdown of CD147 expression was generated as described in Supplementary methods. Forced expression and knockdown of target genes in HCC cells were described in Supplementary methods. In addition, 76 human HCC tissue samples were obtained as previously described [18].

Quantitative real-time reverse transcription PCR, Western blot and immunohistochemistry

RNA extraction, complementary DNA synthesis, quantitative real-time reverse transcription PCR (qRT-PCR) reactions and Western blot were performed as described in Supplementary methods. Primer sequences used in this study are provided in Supplementary Table 3. HCC tissues were processed for immunohistochemical (IHC) staining as previously described [20]. Antibodies and working concentrations are provided in Supplementary Table 1.

Quantification of neutral lipid, triacylglycerols and phospholipids

The lipophilic fluorescence dye BODIPY 493/503 (Invitrogen) was used to monitor the content of neutral lipids in HCC cells as described in Supplementary methods. For quantitative estimation of triglyceride or phospholipids, lipids were extracted from cell homogenates using chloroform/methanol (2:1). The enzymatic assay was then performed using EnzyChrom™ Triglyceride Assay Kits (BioAssay Systems, Hayward, CA) and EnzyChrom™ Phospholipid Assay Kits (BioAssay Systems, Hayward, CA) according to the manufacturer's protocols.

Assessment of fatty acid oxidation

Assessment of FAO in HCC cells was performed as described in Supplementary methods.

Analysis of fatty acids by Gas Chromatography-Time of Flight Mass Spectrometry (GC-TOF-MS)

The contents of free fatty acids in different HCC cells were analyzed by (GC-TOF-MS) as described in Supplementary methods.

Cell proliferation, migration, and invasion assays

Cell proliferation ability was measured according to the protocol described in Supplementary methods. Wound-healing and transwell invasion assays were performed to assess cell migration and invasion ability as described in Supplementary methods.

In vivo assays for tumor growth and metastasis

Nude mice xenograft model was used to assess *in vivo* tumor growth and metastasis as described in Supplementary methods.

Statistical analysis

Unpaired *t* tests were used for comparisons between two groups. Correlations between measured variables were tested by Spearman rank correlation analysis. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software package (version 17.0, SPSS, Inc.).

Results

CD147 silencing significantly decreased lipid contents in HCC cells

To explore whether CD147 is involved in the regulation of aberrant lipid metabolism in HCC cells, we performed both bioinformatic analyses and experimental investigations. First, four public datasets of mRNA expression in HCC tissues were obtained and the KEGG pathway enrichment analysis showed that genes which were significantly correlated to CD147 in